ASPERGILLUS FLAVUS, A. ORYZAE, AND ASSOCIATED SPECIES

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Cultures of fermented food products of the Orient made from rice, other cereals, and soy beans show a number of characteristic types of Aspergillus. Some of these are manifestly only contaminations. A few of them are so closely identified with these food products as to call for comparative study to determine their significance in the fermentation processes under investigation. These organisms are recorded under the names *A. flavus* Link, *A. oryzae* Ahlb., *A. Wentii* Wehmer, and *A. tamari* Kita. Study of cultures from many correspondents and from a wide variety of foodstuffs shows clearly that these forms are not limited to the Oriental fermentation industries but are cosmopolitan. The numerous strains found align themselves into groups of closely related forms which may for convenience be considered here under three series names, *Aspergillus flavus-oryzae*, *A. Wentii*, and *A. tamari*.

ASPERGILLUS FLAVUS-ORYZAE SERIES

The saké industry of Japan is based upon the diastatic power of *Aspergillus oryzae* (Ahlb.) Cohn.[1] The organism as actually used is a well-marked form and its activities have been extensively discussed. Cultures of this species which have been distributed widely from the Centralstelle[2] at Amsterdam show the morphology and culture reactions clearly described by Wehmer[3]. Among large numbers of mold cultures from many sources only one culture which might be confused with the saké organism has been received from a source unconnected with the Oriental fermentation industries.

When, however, numerous cultures from the soy or shoyu industry of Japan and China are brought together, a whole series of forms are found which bridge the gap morphologically between *A. oryzae* as the saké organism and *A. flavus* as described and distributed also by Wehmer (*loc. cit.*, p. 81).

[1] Cohn, F. Ueber Schimmelpilze als Gährungserreger (*A. oryzae*). Jahresb. Schlesisch. Gesellsch. f. vaterland. Cultur **61** (1883): 226–229. Breslau, 1884.

[2] Centralstelle für Pilzkulturen, Roemer Visscherstraat 1, Amsterdam.

[3] Wehmer, C. Die Pilzgattung Aspergillus in morphologischer, physiologischer, und systematischer Beziehung unter besonderer Berücksichtigung der mitteleuropaeischen Species. Mém. Soc. Phys. Hist. Nat. Genève **33**[2]: 1–156. Pls. 1–5. 1901. This paper is commonly cited as Wehmer, Monograph (Monogr.).

Material taken directly from fermenting vats in China by Dr. Yamei Kin, formerly of the Bureau of Chemistry, shows strains of this character. Inoculating material furnished by Dr. Teizo Takahashi for experimental work on the fermentation of soy sauce or shoyu proved to be a member of this series. Dr. Takahashi had selected his strain for this type of fermentation from among several recognized and studied by him in Tokyo. Our thanks are due to Dr. Takahashi for discussing his views upon the group of organisms used by the fermentation industries of his country. These forms showed variations toward the saké organism and others markedly of the "*flavus*" type. All of these strains are regarded by him as varieties of *Aspergillus oryzae*, not *A. flavus*. In the fermenting samples examined, the dominant organism in every case has been nearer *A. flavus* in the sense of Brefeld and Wehmer than *A. oryzae* (Ahlb.) Cohn. The same condition is readily disclosed by cultures from certain of the koji products distributed under the patents[4] of Takamine in which the name *A. oryzae* is used, not *A. flavus*. Although the study of strains widely separated in the series gives easily measurable differences, comparison of large numbers of strains from many sources furnishes intermediate forms which break down the value of such contrasting characters. All of these forms show mixtures of yellow and green color when grown on Czapek's solution agar which, when compared with Ridgway's plates, are found to be closely related. The whole group is found to possess conidiophore stalks and conidia with walls pitted. Stalk walls when examined with low magnification are often recorded as delicately rough, and conidia as delicately rough or spiny. Careful examination with high powers shows these appearances to be due to pits. Upon the ripe conidia the pits, instead of being circular, are commonly elliptical, giving an appearance sometimes designated as areolate.

Variations in length and diameter of stalk, thickness of wall, and number and arrangement of sterigmata are found, but the texture and markings of the walls, the formation, shape, and development of parts appear to link together related forms, hence to have value in characterization. Accuracy in these observations becomes, therefore, essential. Johnston[5] notes discrepancies in the description of the same culture by different workers. We find the same difficulty in our own notes. Cell walls examined with the lower powers, especially the dry objectives, may be recorded as rough or spinulose. The same cell walls examined with the apochromatic objective appear pitted. It has been found necessary to make many examinations of each species or strain studied, both separately and in comparison with other related forms.

The data used in this paper have been obtained by using a Zeiss 3 mm., N. A. 1.30 apochromatic objective with a Zeiss 12 x compensation ocular.

[4] Takamine, J., U. S. nos. 27401; 525.820; 525.823; 525.824.

[5] Johnston, J. R. The entomogenous fungi of Porto Rico. Bull. Board Commrs. Agr. Porto Rico 10: 17. 1915.

The forms reported here have each been restudied several times, some of them at intervals of several years, to determine which characters were variable with conditions of culture and which were stable.

The following cultural descriptions of *A. flavus*, *A. oryzae*, *A. parasiticus*, and *A. effusus* are prepared as typical for races or groups of nearly related strains, which represent fairly widely separated portions of the whole series.

Characterization of A. flavus Link.[6] Colonies on Czapek's solution agar with sucrose, spreading widely, with floccosity limited to scanty growth of a few aerial hyphae in older and dryer areas among the erect crowded conidiophores; sclerotia at first white, then brown, hard, parenchymatous, in a few strains white-tipped, produced abundantly by some strains, scantily by others under undefined conditions, not or rarely by still others; perithecia not found. Conidial areas ranging in color in different races from *sea-foam yellow* through *chartreuse yellow*, *citron green* or *lime green*, to *mignonette green*, *Krönberg's green*, or more rarely to *ivy green* (see Ridgway XXXI. 25″ f,d,b,i,k,m;[7] approximately C. D. C. 270, 271, 266, 252, 253, 257),[8] persistent or changing in very old colonies toward *Isabella color* to *brownish olive* (Ridgway XXX. 19″ l,m), zinc orange (Ridgway XV. 13′), or even *Saccardo's umber* (Ridgway XXIX. 17″ k); reverse (or under side) and agar either uncolored or more or less intensely yellowed, from *pinkish buff*, *cinnamon buff*, to *clay* or even *Saccardo's umber* (Ridgway XXIX. 17″ d, b, to k), or in some cases even darker brown in old and dry cultures. Stalks arising separately from substratum, 400 to 700 μ ,even to 1000 μ long, 5 to 15 μ in diameter, broadening upward, with walls colorless, so pitted as to appear rough or spiny with low magnification, occasionally granuliferous, varying in thickness, gradually enlarging to form a vesicle 10 to 30 or even 40 μ in diameter. Heads in every colony varying from small with a few chains of conidia to very large stellate or columnar masses, or both mixed in the same area (fig. 1, *c* and *d*); small heads with small dome-like vesicles and a single series of a few sterigmata up to 10 to 15 μ by 3 to 5 μ; larger heads partly with simple sterigmata, partly with branched or double series, or with both in the same head; primary sterigmata 7 to 10 μ by 3 to 4 μ; secondary series 7 to 10 μ by 2.5 to 3.5 μ; conidia pyriform to almost globose, from almost colorless to yellow-green, with walls so thickened as to leave circular, elongated, or winding pits, giving a rough or echinulate effect[9] when viewed with low magnification, varying in size in different strains and even in the same culture, frequently 2 by 3 μ, 3 by 4 μ, 4 by 5 μ, or 5 by 6 μ in diameter, or even larger in some strains.

Colonies grow best in starch- and sugar-containing media; some strains fruit at temperatures up to 50° C. Spores survived heating[10] to 57.2° C. for 30 minutes and dry heat at 110° C. for 30 minutes.

This description was originally based upon culture no. 108 received from Amsterdam and identical with no. 3526 obtained directly from Wehmer.

[6] This characterization is revised and extended from the form furnished to Dr. John R. Johnston and published by him (*loc. cit.*).

[7] Ridgway, R. Color standards and color nomenclature. Washington, D. C., 1912.

[8] Klincksieck, P., and Valette, T. Code de couleurs. Paris, 1908.

[9] "Areolate" of Johnston.

[10] Thom, C., and Ayers, S. H. Jour. Agr. Res. 6: 153. 1916.

This organism in Czapek's solution agar is *Krönberg's green* without color in the substratum and without sclerotia. The following supplementary cultures are cited: no. 3557.9 from corn (isolated by Clawson) differs by the production of sclerotia, and yellow color in the substratum; no. 128, after many transfers identifiable with no. 108, produced a sclerotium—former resembling no. 3557.9; no. 2773 from Demerara is *mignonette green*, produces abundant sclerotia and yellow color in reverse. In general, sclerotium formation is found correlated with the production of yellow color in the submerged mycelium and with reduced intensity of green in the conidial area.

Numerous strains with the same cultural characters have been obtained from many sources. The considerations leading to the retention of the name *A. flavus* for these forms are discussed later under "nomenclature."

A. ORYZAE SERIES

Aspergillus oryzae has been generally accepted as a valid species. It is characterized as a group of varieties by Costantin and Lucet.[11] In the typical form represented by the cultures and descriptions of Wehmer, the species is readily separated from cosmopolitan forms of *A. flavus*. In the Oriental industries in which it has been long used, the separateness of this form is largely lost. It becomes, therefore, a gigantic race in a group in which other members possess the same habits, the same essentials of structure, but differ slightly in color and greatly in size. Growth upon different substrata produces great differences in the appearance of colonies. The fruiting stalks on Czapek's solution agar are commonly 2 to 3 mm. in length, much longer on richly organic media, and are reported by Takahashi[12] to attain a length of 20 to 30 mm. upon special rice media upon which the stalks of *A. flavus* reach a length of 5 m to 8 m.

In contrast with *A. flavus* as already described, the following characterization from cultures is proposed.

A. oryzae (Ahlb.) Cohn. Colonies on Czapek's solution agar spreading broadly, pale greenish yellow (at its greenest about *lime green* to *mignonette green*. Ridgway, *loc. cit.* XXXI. 25″ YG–Y), with the green fading early to leave yellowish brown shades; sclerotia dark, produced occasionally, few and in clumps; mycelium and agar uncolored; stalks 2 to several millimeters long, up to 20 to 25 μ in diameter; heads both large and small in the same culture, predominantly large, globose, and radiate rather than calyptrate; sterigmata most commonly 1-series, occasionally 2-series, primary sterigmata up to 8 to 12 by 5 μ, secondary when present 7 to 10 by 3 μ; conidia pyriform, colorless to very slightly yellow with walls so thickened as to leave circular, elongated, or winding pits[13] giving a rough or echinulate

[11] Costantin, J., and Lucet, L. Recherches sur quelques Aspergillus pathogènes. Ann. Sci. Nat. Bot. IX, 2: 119–171. 1905.

[12] Takahashi, T. Preliminary note on the varieties of *Aspergillus oryzae*. Jour. Coll. Agr. Tokyo 1: 137–140. 1909.

[13] "Areolate" of Johnston, *loc. cit.*

effect when viewed with low magnification, varying in size, predominantly larger than *A. flavus*, 3 by 4 μ, 4 by 5 μ, 5 by 6 μ, 6 by 7 μ, occasionally 5 to 6 by 8 to 10 μ.

This description is primarily based upon culture no. 113 from Amsterdam. The same form has been isolated at various times from fermented products (once from a Brazil nut), and received in exchange from various workers. A series of 3 varieties were first studied by Takahashi[14] in 1908. This work was continued with the accumulation of a series of strains under this name which have been furnished to us for study. These are lettered[15] with the alphabet from A to P, then skip to Z, and all are regarded as *A. oryzae*.

These cultures were transferred and grown under conditions as uniform as possible in Czapek's solution agar. The resulting colonies were arranged into a series to correspond with our conception of the relationships involved. This may be tabulated as follows:

Takahashi strains arranged in order of appearance of colonies:

H. White, nearly sterile, floccose mycelium.

O. Slight fruiting, predominantly yellow.

B. Increase of fruiting, still a floccose colony. Near *A. giganto-sulphureus*.

G. Further development of fruit at expense of floccosity.

Z. Long stalks, large heads, floccose effect.

D. Mycelium and long-stalked fruits, both evident.

N. Abundant stalks and heads, no green color. Near *A. perniciosus*.

F. Close resemblance to no. 113, *A. oryzae* of Wehmer.

I. Short-stalked, form otherwise near no. 113.

A. Still shorter.

M. Same morphology, green color more prominent.

L. Slightly paler form with shorter stalks.

C. Close to no. 108, *A. flavus* of Brefeld and Wehmer.

P. Shorter stalks (crowded; more slender type), green passing to reddish brown. Near *A. micro-virido-citrinus*.

J., K., E. } Aberrant forms { suggesting the same line of transformation from strain C as is found in *A. effusus*, though differing from previously examined representatives.

Similarly, Z, G, B, O, and H are progressive reductions from the *A. oryzae* type found in strain F.

This table shows strain F to represent approximately the form already described as *A. oryzae* (no. 113). With almost entire loss of green color and progressively increasing floccosity, strains N, D, G, B, O, and H end at H in almost complete loss of conidium production. The absence of all green color

[14] *Loc. cit.*

[15] The lettering is maintained to correspond with Dr. Takahashi's usage in his own papers. Takahashi, T., and Yamamoto, T. On the physiological differences of the varieties of *Aspergillus oryzae* employed in the three main industries in Japan, namely saké, shóyu, and tamari manufacture. Jour. Coll. Agr. Tokyo 5: 153–161. 1913.

makes strain N conspicuous as a variation which might easily be regarded as a distinct species. From F the strains I, A, M, and L grade in appearance toward C, which is closely similar to *A. flavus* as already described (no. 108).

The tendency toward floccosity and toward quick disappearance of green color appears again in L.

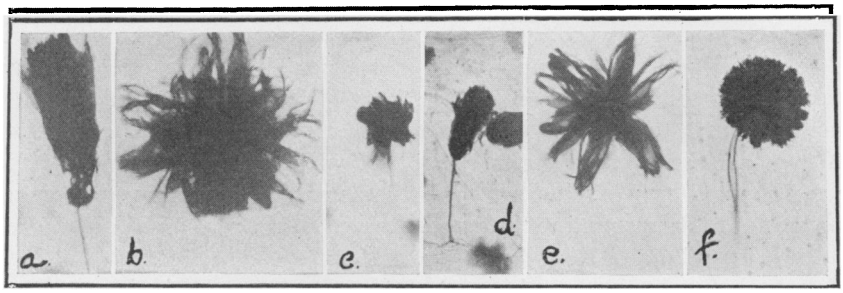

FIG. 1. The photomicrographs composing this figure represent the wide variety of heads in a species and in a strain. The magnifications are various and are not given. *a.* Calyptrate head of *A. tamari*. *b.* Radiate and large head of *A. tamari*; the same strain as *e.* *c.* Head of no. 108, type of *A. flavus*. *d.* Columnar head of no. 129; a delicate, pale form of *A. flavus*. *e.* Radiate head of *A. tamari*, showing a less compact structure than *b.* *f.* Globose head of *A. Wentii*, with heavy-walled stalk.

P is a more slender, delicate form than C, with crowded stalks, and also loses its color quickly.

A. oryzae var. *basidiferens* Costantin and Lucet[16] differs from *A. oryzae* as described by Wehmer in having both primary and secondary sterigmata. Since the authors of this variety had no other cultural experience with *A. oryzae*, and since all cultures we have seen appear to show this character, it seems best to us to introduce this observation as an emendation to the description of *A. oryzae* instead of recognizing the validity of a variety.

A. pseudoflavus Saito[17] appears to represent some one of the races intermediate in length of stalk between *A. flavus* and *A. oryzae* but having the color, usually simple sterigmata, and size of conidia found in *A. oryzae*. *Aspergillus micro-virido-citrinus* Costantin and Lucet[18] differs from *A. pseudoflavus* only in its smaller conidia. *A. gymnosardae* Yukawa[19] was found upon the fermented fish product, katsuobushi, in Japan. The description clearly marks it as also intermediate in structure between *A. flavus* and *A. oryzae*. We have not been able to identify either of these forms with certainty, although we have had several strains in culture which occupy such an intermediate position.

Similarly, another strain appears in our collection once from America

[16] *Loc. cit.*, p. 167.

[17] Saito, K. Centralbl. Bakt. II, **18**: 34. figs. 15–18. 1907.

[18] *Loc. cit.*, p. 158.

[19] Yukawa, M. Jour. Coll. Agr. Tokyo 1: 362. Tab. XVIII, figs. 1–7. 1911.

(no. 129), and once contributed by Hanzawa from Japan. This form in culture is *citron green* to *lime green* (Ridgway XXXI. 25″). It has short, crowded stalks like *A. fumigatus* with small heads, with mostly a single series of sterigmata and conidia predominantly small, 3 to 4 μ, few reaching 5 to 7 μ in diameter.

A. parasiticus Speare. Speare[20], working in Honolulu, found one of these forms parasitic upon the mealy bug of sugar cane (*Pseudococcus calceolariae* Mask), and described his strain as *A. parasiticus*. A culture with the same characters was isolated by one of us from mealy bugs obtained from Demerara; another culture was isolated from cane sugar in New Orleans by Kopeloff. However, other strains of the *flavus* group were isolated by Johnston[21] from mealy bugs in Porto Rico; reinfection experiments by Johnston, while not conclusive, established a presumption of infectiousness as a strain or race character unconnected with the specific morphology of Speare's *A. parasiticus*.

Speare's organism when grown on Czapek's solution agar differs from the commoner forms of *A. flavus* in its predominantly greener color (near *ivy green* Ridgway XXXI. 25″ m), in short stalks usually 200 to 400 μ long, in heads with usually a single series of sterigmata 7 to 10 μ long by 3 to 5 μ. No sclerotia have been seen. The mycelium is uncolored. Otherwise the characters are those of the *A. flavus* group.

A. effusus Tiraboschi.[22] Cultures of a cottony, floccose type have been obtained from widely separated sources (no. 130 from Dr. B. F. Lutman, Burlington, Vermont; no. Sc. 171 in corn meal from Indiana; no. 2750 isolated by Johnston from mealy bugs in Porto Rico). Superficially these cultures show little relation to *A. flavus*. Microscopic examination of heads and spores, however, shows close relationships.

Characterization of A. effusus: Colonies on Czapek's solution agar with sucrose, broadly spreading, effused floccose, or cottony, white becoming slowly dirty yellowish or in restricted areas greenish yellow; reverse and agar yellowish. Stalks either *A. flavus*-like arising directly from the substratum, up to 500 μ long and frequently with large radiate heads, or predominantly in the form of branching, trailing, thick-walled hyphae, each segment consisting of a long, thick-walled, fertile cell bearing a perpendicular branch (stalk) usually less than 100 μ long by 5 to 10 μ in diameter, with walls pitted and sometimes granuliferous, bearing usually columnar heads; vesicles in small heads up to 20 μ in diameter, occasionally larger, sterigmata in one series, 6 to 10 μ by 3 to 5 μ, mostly on apex only of vesicle; larger heads with either simple or branched sterigmata as in *A. flavus*. Conidia pitted as in *A. flavus*, pale yellow, rather thin-walled, pyriform to globose, varying in size in the same culture from 3 by 4 μ to 5 by 7 μ. Neither

[20] Speare, A. T. Fungi parasitic upon insects injurious to sugar cane. Hawaiian Sugar Planters Exp. Sta. Path. and Phys. Ser. Bull. 12: 30. 1912.

[21] *Loc. cit.*, p. 15.

[22] Tiraboschi, C. Atti Terzo Congresso Pellagrologico Italiano, p. 142. Milano, 1906; diagnosis in Annali di Botanica 7: 16. 1908.

sclerotia nor perithecia were found. Colonies grew well in all common media, grew better at 37° C. than at 20° C., liquefied plain gelatine (gelatine in distilled water) with yellow color in the liquid.

The puzzling appearance of these cultures led to studies in morphology and to extensive experiments scattered over nearly ten years. Comparative study of all species obtainable shows that the stalk throughout the genus Aspergillus originates as a mycelial cell transformed into a spore-producing organ. The cell enlarges in diameter and its wall becomes thickened. The stalk arises as a branch approximately perpendicular to the course of the original cell which remains in the hypha as a kind of foot. Usually the stalk, beginning with the diameter of its foot cell, broadens upward and lengthens, hence becomes many times the size of its foot. In *A. effusus* the foot cell is frequently very long, branching and connected with other foot cells to form a trailing, fertile hypha from which the stalks arise as short branches. Selective transfers from Lutman's strain (no. 130) showed the possibility of separating a race which appeared to be the usual form of *A. flavus* and another in which the heads were borne only on the trailing type of fertile hyphae among cottony white masses of sterile hyphae. The hypotheses of symbiosis and of parasitism were both tested through many transfers without result. The heads on all series of cultures maintained the essential morphology of *A. flavus*, although the colony characters diverged widely. More recently, a transfer was made from a stock culture several months old which was grown upon Czapek's solution with the addition of 5 percent sodium chloride, 10 percent sucrose, and 3 percent agar. The original strain (Lee 108) had maintained the cultural appearance of *A. flavus* as received from Wehmer, through successive transfers for about four years. This transfer produced a floccose type of colony with some *A. flavus* type of fruiting at the edges. Transfer from the whitest areas in this culture produced the typical white colony described above; transfer from an area showing few small heads produced a mixed colony; transfer from selected heads appearing to be *A. flavus* gave a pure *A. flavus* colony. All of the experimental work supports the hypothesis that the floccose types represent mutants from the typical *A. flavus*, which were probably induced in the last experiment in similar manner to the mutations of *A. niger* as described by Schiemann.[23]

The description given by Tiraboschi (*loc. cit.*) for *A. effusus* probably applies to these cultures. *A. effusus* was isolated by Tiraboschi from spoiled corn products.

Nomenclature. *Aspergillus flavus* was first described by Link[24] in

[23] Schiemann, E. Mutationen bei *Aspergillus niger* van Tieghem. Zeitschr. Indukt. Abstam.- u. Vererbungslehre **8**: 1–35. 1912.

[24] Link, H. F. Observations in: Ordines plantarum naturales. Gesellschaft Naturforschender Freunde zu Berlin, Magazin 3, p. 16. 1809. This is usually cited Link. Obs. p. 16. 1809. The description in full follows: "Caespitibus laxis, floccis albis erectis, capitulis junioribus albis, adultioribus flavis. Frequens in plantis siccis herbariorum."

terms vague enough to baffle any attempt at certain identification. The habitat given was herbarium specimens. Our own search over many lots of moldy plants in herbaria, together with interpretation of the name *flavus*, suggested that some one of the *Aspergillus herbariorum-repens-Amstelodami* series in the sense of Mangin[25] was the basis of Link's description. Specimens have been actually found in several series of exsiccatii labeled *A. flavus* but clearly consisting wholly of *A. repens*. However, Link records his acquaintance with the green Aspergillus of the herbarium under the name *A. glaucus*. The universally distributed yellow perithecial forms which were later connected with the common green forms by De Bary were known to Link under the name Eurotium. Clearly, then, Link believed that he had some organism which he found commonly upon badly dried herbarium specimens and which was yellow enough in contrast to *A. glaucus* to justify the name *A. flavus*. The conidial forms of the *A. glaucus* group do not show a yellow color factor. Following De Bary,[26] there is, moreover, the widespread use of this specific name *A. flavus* Link for our series of yellow-green forms which are universally distributed.

Wilhelm,[27] Schroeter,[28] and Wehmer[29] base their use of this name upon Brefeld's specimens distributed as no. 2135 in Rabenhorst's[30] Fungi Europaei. A comparison of Brefeld's specimens with a culture obtained in Wehmer's laboratory in 1905 and still maintained by us in culture shows them to be morphologically identical. This strain agrees with the characterization of *A. flavus* in Wehmer's Monograph. Costantin and Lucet[31] reach the same general conclusion without record of having seen the specimens, and add the comment that this identification constitutes the perpetuation of a tradition that this particular strain is *A. flavus* Link. This identification is promptly discarded by them and its name changed to *A. Wehmeri* Cost. et Lucet.[32]

With the study of the culture from Wehmer as a basis, the distribution of this and closely related strains has been followed for about ten years. Numerous cultures have been isolated and compared from diverse sources. Molds with essentially this morphology have been sent to us in series of soil cultures made by Esten and Mason in Connecticut, by Miss Dale in England, by Johnston in Porto Rico, by Waksman in New Jersey, by McBeth and Scales in Washington, by Werkenthin in Texas, and by Hartley from coniferous seed beds in Kansas. They have been isolated by us many

[25] Mangin, L. Ann. Sci. Nat. Bot. IX, 10: 303–371. 1909.

[26] DeBary, A. Beiträge zur Morphologie der Pilze. III^te Reihe, 2^te Abt., p. 20. 1865.

[27] Von Wilhelm, K. A. Beiträge zur Kenntniss der Pilzgattung Aspergillus. Inaug. Diss. Strassburg. Berlin, 1877.

[28] Schroeter, J. Cohn's Kryptogamenflora von Schlesien 3^2: 216. 1893.

[29] *Loc. cit.*, p. 81.

[30] Rabenhorst. Fungi Europaei Edit. Nov. ser. II. 1875.

[31] *Loc. cit.*, p. 152.

[32] *Loc. cit.*, p. 169.

times from miscellaneous foodstuffs, especially the cereals both as whole grain and as milled products, and more recently have been found abundantly in the soy-bean fermentation products of China and Japan.

Members of this series have been reported in the study of infections in the human ear. Certain of these forms have produced lesions and death when injected into experimental animals. Costantin and Lucet[33] review the literature of such pathogenicity and offer a key to species based upon their review of injection experiments with rabbits and fowls. The structural characters cited by them represent fairly well the range of variation within the group. Sclerotium formation is used to separate *A. flavus*, attributed by them to Wilhelm, from the other forms. In our experience sclerotium formation is not limited to any morphological section of the group. Moreover, *A. Wehmeri* of Costantin and Lucet is a manuscript species based upon *A. flavus* of Wehmer's monograph. It was not studied by them in culture. Both Wilhelm and Wehmer based their use of the name upon the usage of Brefeld as determined by examination of the same cultural material distributed by Rabenhorst.[34] We have seen this material, and it corresponds satisfactorily with the characters given by Wilhelm and Wehmer. If the name *A. flavus* is to be held in the sense of Wilhelm, *A Wehmeri* is clearly a synonym.

A. flavescens of Wreden[35] was not cultivated. The size of the spores and the coloration of the stalk reported caused us to believe that it belonged elsewhere.[36] Wehmer[37] cites Lichtheim as having compared *A. flavescens* with *A. flavus* Link as understood by Brefeld and having found them identical. Lichtheim, however, uses the name as interpreted by Eidam, which is probably but not necessarily identical with the usage of Wreden. Certain organisms from ulcerated ears clearly belonged to this series. The morphology given by Costantin and Lucet for *A. Siebenmanni* and *A. microviride-citrinus* is not uncommon in cultures from the group except as to color. It will be shown later that the green factor in colony color is suppressed when fermentable carbohydrates are omitted from the substratum.

The range of morphology cited by Costantin and Lucet was assumed by them to establish a presumption of pathogenicity to warm-blooded animals for the whole group. The infection experiments reported from different sources were intravenous with positive lesions. It is noteworthy that they found their *A. oryzae* var. *basidiferens*[38] to be pathogenic also to the rabbit by the same kind of inoculation. This is consistent with a common morphology in *A. flavus* and *A. oryzae* as considered in this paper. Double sterigmata, used by Costantin and Lucet as varietal characters, are not

[33] *Loc. cit.*, pp. 151–163.
[34] *Loc. cit.*, no. 2135. 1876.
[35] Compt. Rend. Acad. Sci. Paris **65**: 368. 1867.
[36] Thom, C., and Church, M. B. Amer. Jour. Bot. **5**: 100. 1918.
[37] Wehmer, C. Centralbl. Bakt. II, **2**: 148. 1896.
[38] *Loc. cit.*, p. 167.

the exception but the rule in the saké organism in which only occasional cultures show only simple sterigmata. In the rice and soy fermentation industries of Japan the workman's eyes, ears, nose, throat, and skin abrasions are constantly exposed to Aspergillus spores. Dr. Takahashi and Dr. Kita (personal communications), however, report absolutely no infections. Our cultures show a wide range of varieties of the *Aspergillus flavus* series to be present. Intravenous injection, doubtless, has value in demonstrating the possible activity of an organism when so inoculated, or perhaps in lesions already established by other agencies, without proving active pathogenicity.

Wreden and Siebenmann use the name *A. flavescens* for organisms found in the human ear. Wreden's description lacks essentials for identification perhaps, but Lichtheim certainly had an organism of this group from infected ears. Siebenmann, using the same name, gives details which definitely ally his form with either *A. flavus* or *A. tamari* (see discussion of *A. tamari* later). Wilhelm clearly has a sclerotium-producing strain closely allied to the material studied by Brefeld. Wehmer, whose organism we have in culture, had a different but closely related strain which rarely if ever produces sclerotia. Costantin and Lucet[39] appear also to have had but one strain of the same series, which they described as *A. micro-virido-citrinus*. Cultural observations limited to single strains in a group varying as widely as this may easily lead workers unacquainted with other material to believe they have distinct species. When comparison of hundreds of cultures from separate sources has bridged the gap between these forms, it is doubtful if any effort to maintain such species is desirable.

There appears to be no valid reason for rejecting the name *A. flavus* for the cosmopolitan organism studied by Brefeld and Wehmer and as tentatively covering many strains with minor variations from such a type. *A. oryzae*, *A. parasiticus*, and *A. effusus* are morphologically recognizable varieties or species which are certainly closely related to the cosmopolitan group of which the organism described by Brefeld and Wehmer and believed by them to be *A. flavus* Link may be called the type.

In reaching this conclusion, many series of cultures were made with a large number of strains selected to represent the widest range of variation found in our collection. These cultures included an extensive variety of culture media; the bark of Castanea, Liriodendron, Platanus, and Tsuga, oatmeal agar with and without sugar, potato plugs, beef extract peptone agar, egg albumen, beef plugs, loam, rice, cooked soy beans mixed with ground and roasted wheat, Czapek's solution with cerealose instead of sucrose. The following paragraphs describe some points observed which seem to be worthy of note.

Czapek with 50 percent saccharose: Twenty-seven strains of *Aspergillus flavus* grown on Czapek solution agar containing 50 percent saccharose grew for all practical purposes the same as if on unmodified Czapek solution

[39] *Loc. cit.*, p. 158.

agar. In 3 strains of *A. effusus*, fruiting was increased and the conidial areas and the reverse were *citrine* in color. The two strains of *A. oryzae* were more brownish in color and the conidiophores were short as compared with the growth of the same strains on the standard Czapek solution agar. *A. terricola* var. *Americana* grew sparsely on this medium.

Fish agar (halibut): Nine strains of *A. flavus* when grown on fish agar for two months developed only a few brown heads; nine other strains of the same species developed only white mycelium. *A. oryzae* and *A. effusus* also did not fruit. *A. tamari* and *A. Wentii*, however, showed fruiting at first *old gold* in color and scarcely spreading beyond the mark of the streak.

Beef plugs: Plugs of fresh beef were cut with a cork borer and placed in tubes ordinarily used for potato plugs. The sterilization was fractional. At the end of two weeks six of the more common strains which develop conidial areas near in color to *Krönberg's green* on Czapek solution agar were *olive ocher* (Ridgway XXX. 21″) on the beef, four others ran through *olive ocher* to *old gold* (Ridgway XVI. 19′ i); three strains changed from *olive ocher* to other tints and shades of orange and yellow; one strain never developed any deeper color than *deep colonial buff* (Ridgway, XXX. 21″ b). All the green color was, therefore, eliminated from these strains of *A. flavus* when grown on cooked beef, with the exception of one strain which became *lime green* after it had appeared *olive ocher*. The early growth of *A. parasiticus* was at first *mignonette green* and later *olive lake* (Ridgway XVI. 2′ i), a shade with no green; a yellow green strain (no. 129), possibly *A. microviride-citrinus*, corresponding with *A. terricola* of the brown series, was *colonial buff* (Ridgway XXX. 21″); and strains of *A. effusus* at the end of two weeks were *chamois* (Ridgway XXX. 19′ b). No green color was exhibited in the whole group.

Plain agar (bacteriological): The *A. flavus* group when planted on plain agar produced color practically as when grown on beef plugs. The green factor was not suppressed as completely, however. It was more evident during the first few days of growth, and seemed to disappear except in the same instances noted under beef plugs.

Synthetic agar (Currie's):[40] Thirteen of the *A. flavus* strains and *A. parasiticus* developed the green color more intensely with a reduction of yellow, when grown on this agar. They developed such shades and tints as *Kildare green* (Ridgway XXI. 29″ b), *Rainette's green* (Ridgway XXI. 27″ i), *cress green* (Ridgway XXI, 29″ k), etc. Six similar strains grew as if on Czapek solution agar, as did also *A. effusus*.

In these experiments the colors reported range from mixtures of yellow and orange to various combinations of yellow and green. The reversible factor appears to be green. Kita[41] reports similar observations. In

[40] $(NH_4)H_2PO_4$, 2.0 gms.; KCl, 0.2 gm.; $MgSO_4$, 0.1 gm.; cane sugar, 30 gms.; agar, 15 gms.; H_2O, 1 l. Formula used by Dr. J. N. Currie.

[41] Kita, Gen-itsu. Ueber die Konidienbildungsfaehigkeit einiger Varietaten des *Aspergillus Oryzae*. Original Communications, 8th International Congress of Applied Chemistry 14: 95.

describing *A. pseudoflavus*, Saito[42] observed that exposure to ammonia would destroy the green color entirely, leaving yellow or yellowish brown. Subsequent exposure to vapor of acetic acid restored the green color to this colony. The test used by Saito was applied to *A. flavus* (no. 108, the Wehmer strain), *A. oryzae* (the saké organism), and *A. parasiticus*, which represent the widest range of differences in our collection. Each of these strains gave the reactions described by Saito for *A. pseudoflavus*. The test was repeated with hydrochloric acid substituted for acetic acid. The correlation of the green color with the acid was clearly brought out. These strains grown in Czapek's solution agar are, respectively, *A. oryzae* about *lime green*, *A. flavus* near *Krönberg's green*, and *A. parasiticus* close to *ivy green*, all in column 25″, Plate XXXI of Ridgway's tables. When exposed to the fumes of hydrochloric acid the green color was intensified, reaching colors given in column 29″ of the same plate with the deepest areas reaching the same intensity, *cress green*. When the same cultures were exposed to ammonia, all of the green disappeared, and the colors remaining corresponded with combinations in column 21″, Plate XXX, varying from *deep colonial buff* to *olive* in the deepest areas.

In the experiments previously described the green colors are found present in marked degree only upon media containing sucrose or some other fermentable carbohydrate. In cultures upon beef, fish, egg, and soy beans, which lack carbohydrates or are very poor in fermentable carbohydrates, the greens were absent or nearly so throughout the series. In the mixtures of fermentable carbohydrates and proteins there are evidently simultaneous acid and alkaline fermentations which tend to neutralize each other as described by Ayers and Rupp.[43]

The early development of conidia always shows some development of green in such cultures. When litmus is used in the culture medium, these early stages are always accompanied by the acid or red reaction. Many such cultures eventually lose all their green color, but this loss is always preceded by change in reaction. If a series of these strains grown upon a single medium show different shades of green, these shades of green are thus indications of the relative acidities reached by the culture. Some correlation between the typical color shown by a colony and the progressive changes in the reactions of the substratum, and possibly even of cytoplasm, is indicated.

The substratum influences the development of such saprophytic fungi in numerous directions. The gross appearance of a pure mold culture may be entirely altered through the influence of the medium on which the fungus is growing. The dimensions of certain structures in an Aspergillus

[42] Saito, K. Microbiologische Studien über die Zubereitung des Batatenbranntweines auf der Insel Hachijo (Japan). Centralbl. Bakt. II, **18**: 30–37. figs. 1–22. 1907.

[43] Ayers, S. H., and Rupp, P. Simultaneous acid and alkaline fermentations from dextrose and the salts of organic acids respectively. Jour. Infec. Dis. **23**: 188–216. 1918.

may be altered by a change in the medium, while those of other structures may remain constant as long as the medium is not totally inadequate or does not contain deleterious substances. Aborted or unrecognizable types of structure result from conditions positively inhibitive for normal growth. Conidial growth, sclerotia, or perithecia, each may be totally or in part suppressed or their production may be stimulated by the nutrient provided. The structure and markings of the stalk wall, general shape, markings, and range of size in conidia are fairly stable within the strain or species and fall within certain limits which for practical purposes do not vary. The length of the stalk, diameter of the vesicle, the dimensions of the primary sterigmata, and, within limits, the spore measurements are influenced by the substratum. Wall markings cannot be said to vary with the nutrient supplied, although their conspicuousness varies slightly from culture to culture doubtless through the pressure of several factors. The alterations in these latter structures are never permanent. They are dependent entirely on the substratum. Certain media stimulate in such fashion as to cause an increase in dimensions, others a dwarfing. The majority of culture media cause each strain to develop to a size falling within fairly well-defined limits.

Aspergillus Wentii and Related Forms

Aspergillus Wentii was described by Wehmer[44] and has been widely distributed in culture from the Centralstelle at Amsterdam. Extensive cultural studies show the species to differ from the characterization given by Wehmer in the quite general presence of both primary and secondary sterigmata. Identity of the Amsterdam strain with Wehmer's material is hardly questionable. Cultures with the same morphology have been found by us upon moldy corn grains, upon moldy cotton cake from Georgia, (4230) within a temechee nut from Brazil, upon cubebs from Singapore, and received (4204.16c) from China through the kindness of Mr. Chung, from Hanzawa (4291.32) in Sapporo, (4186.34) from Panama collected by Dr. Thaxter, from Oregon soil (4078.0–5) collected by Waksman. One culture was received from Ohio Experiment Station, one from Prof. R. A. Harper. Although these forms differ in details of reaction and appearance, the morphological characters found mark them as a natural group.

Characterization of A. Wentii Wehmer. Colonies on Czapek's solution agar with cane sugar, deeply floccose, spreading, with sterile hyphae white or yellowish, and with heads white at first, changing through *cream*, *cream buff*, *honey yellow*, *old gold*, to *light brownish olive*, *medal bronze*, or in old cultures sometimes *snuff brown* (Ridgway, column 19, Plates IV, XVI, XXX, and Plate XXIX 15″ K; recorded as *coffee-brown* to *chocolate brown* by Wehmer), and in some strains producing large masses of aerial mycelium which in tubes may fill the lumen 3 cm. above the substratum; reverse of colony yellowish at first, becoming reddish brown when old; agar frequently

[44] Wehmer, C. Eine neue technische Pilzart Javas. Centralbl. Bakt. II, 1: 150. 1895.

colored yellow; stalks 2 to 3 mm. or up to 5 mm. long, commonly 10 to 12 μ or sometimes up to 25 μ in diameter, inconspicuously 1- to 2-septate, with walls colorless, up to 4 μ in thickness, and smooth, often studded with droplets in young cultures, enlarged at tips to vesicles widely varying up to 80 μ in diameter; heads large, yellow to brown, stellate (or globose fig. 1, f); sterigmata usually in two series, primary varying greatly, 6 to 8, occasionally to 15 μ by 3 to 5 μ, in extreme cases up to 60 μ by 8 to 10 μ; secondary 6 to 8 by 3 μ (a single series is recorded by Wehmer 15 by 4 μ). Conidia pyriform to globose, usually about 4 by 5 μ, less commonly up to 5 to 5.5 μ by 5 to 6 μ (4.2 to 5.6 μ, Wehmer), with walls thickened to leave pits or furrows on the surface arranged roughly lengthwise of the spore chain, frequently appearing smooth or nearly so with low magnifications, commonly more or less plasmolyzed when treated with 95 percent alcohol.

Perithecia not found. Sclerotia limited to more or less undefined masses of thick-walled cells occurring occasionally, not uniformly. Cultural optimum below 37° C. in all strains tested. Gelatin liquefied in cultures, both with and without sugar.

The Java culture originally sent by Went to Wehmer was used in rice and soy fermentation on that island by Chinese workmen. The strains since found resemble the Amsterdam strain in their range of color changes, smooth, thick-walled stalks without pits, stellate heads, double sterigmata, and in the *A. flavus*-like marking of the conidial wall. The mass of sterile mycelium above the colony in typical test-tube cultures of the organism, as described by Wehmer, is present in the Amsterdam strain, but lacking or only partially or occasionally present in some of the strains. This overgrowth of mycelial masses with fruiting at several levels through a considerable period becomes more prominent upon potato plugs. A gradation from the type strain of Wehmer to those lacking this character in Czapek solution agar cultures, combined with the common structural characters cited, justifies the extension of our idea of *A. Wentii* to include these forms at least as varieties of a widespread natural group.

Inui[45] described *A. perniciosus* as found in awamori-koji without giving details of stalk and spore markings but comparing the stalks with those of *A. Wentii* and *A. luchuensis* both of which we have in culture. *A. perniciosus* belongs probably in the group with *A. flavus* and *A. oryzae* on account of the data given as to color and habit, which correspond to certain of the variant strains contributed by Dr. Takahashi.

A. TAMARI AND ALLIES

A second brown series of forms is more closely associated in occurrence and in habit with *A. flavus* and its allies than is *A. Wentii*. Many cultures in this series have been obtained in forage and feeding stuffs, from the Oriental soy fermentations, upon food, in soil, and growing as laboratory contaminations. Cultures have been thus obtained from China, Japan,

[45] Inui, T. Jour. Coll. Sci. Imp. Univ. Tokyo 15: 473. 1901.

and South America as well as from many points in the United States. These brown forms are characterized by absence of true green in color, by stalks prominently pitted especially toward the apex, and by conidia tuberculate at the distal end in the chain, rough, showing on detailed examination firm, fairly thick, and not pitted inner walls, thin, vesicular outer walls fitting rather loosely over masses of branching, more or less irregularly-arranged bars of yellow-brown substance. In size of colony, habit, and appearance aside from color, these forms resemble *A. flavus*. In the markings of conidia they suggest *A. niger*.

Characterization of A. tamari Kita (from our culture no. 4235. I–2). Colonies on Czapek's solution agar with cane sugar spreading broadly, with vegetative hyphae mostly submerged, with fruiting areas at first colorless, then passing through orange-yellow shades to brown in old colonies (variously *Isabella color*, *light brownish olive*, *buffy citrine*, *medal bronze*, or *raw umber*. Ridgway *loc. cit.*, column 19, Plates XXX, XVI, IV, and column 17, Plate III), not showing true green; reverse uncolored or occasionally pinkish; stalks arising from submerged hyphae, up to 1 to 2 mm. in length, becoming several millimeters in length upon corn or other concentrated media, 10 to 20 μ in diameter, increasing in diameter toward the apex and passing rather abruptly into vesicles, with walls rather thick, 1 to 2 μ, becoming abruptly thinner at the base of the vesicle, pitted more prominently in upper than lower half (often appearing as rough or echinulate with low magnifications) and frequently showing irregular thickenings within; vesicles 25 to 50 μ in diameter with fairly thin walls which frequently crush in mounts; heads varying greatly in size in the same fruiting area, from more or less columnar to nearly but not completely globose (fig. 1, *a*, *b*, and *e*), and up to 350 μ in diameter, with radiating chains and columns of conidia; sterigmata, one series in small heads, two series in large heads, primary commonly 7 to 10 by 3 to 4 μ, becoming 20 to 35 μ long in gigantic heads upon corn; secondary 7 to 10 by 3 μ; conidia more or less pyriform toward globose, tuberculate especially at the distal end in the chain, 5, 6, occasionally up to 8 μ in diameter, rough from prominent masses and bars of orange-yellow coloring matter deposited under the loose outer wall upon the firm inner wall. Sclerotia occasionally produced.

These forms are widely distributed and resemble *A. Wentii* in color but have the gross morphology and habits of *A. flavus*. Their conidia show color bars resembling those of *A. niger* in formation and in solubility in water.

In examining the exsiccati, collections have been repeatedly found upon corn grains (*Zea Mays*) with the color and conidial markings of *A. tamari*, but with long stalks whose thick walls obscured the pitting except close to the head and with primary sterigmata 20 to 30 by 5 to 6 μ and larger conidia. Cultures from another strain (S_3 from sardine paste) when grown upon Czapek's solution agar showed vesicles about 35 μ in diameter, primary sterigmata 8 to 14 by 3 to 5 μ, and conidia 5 to 6 μ in long axis. When grown in unsterilized, clean corn for two weeks, this organism showed vesicles 100 μ in diameter, primary sterigmata 25 to 35 by 5 to 9 μ, and conidia 8 to 9 μ in long axis. The secondary sterigmata seem to vary much

less under such differences of environment than the primary sterigmata. Transfer from the corn back to Czapek's solution agar gave the original measurements. Such cultural experiences emphasize the necessity of using a standard medium as the basis of comparative studies of saprophytic organisms and destroy all faith in the significance of measurements to the fraction of the micron from miscellaneous cultures. At best a range of measurements must be allowed for in the most carefully standardized culture work.

Cultures with this group morphology vary in shades of color enough to separate different strains when observed in parallel culture. A ten-day petri-dish culture (no. 3565) showing its outer zone *maize yellow*, intermediate zone *orange-citrine*, and deepest area *medal-bronze* (Ridgway *loc. cit.*, IV. 19 f, k, and m) was inverted over a dish of hydrochloric acid. The shade of mixed yellow and orange quickly changed to *Saccardo's olive* (Ridgway III, 19 m). The petri-dish culture was then placed over a dish of strong aqua ammonia and quickly changed toward a shade between *raw umber* and *Brussels brown* on the same plate (Ridgway III). This color approximates that of very old cultures of members of this series which, in common with those of *A. flavus*, become more alkaline with age. The response in *A. tamari* is not as conspicuous as the change in green shades of the *A. flavus* group, but clearly indicates that differences in color of the same culture at different ages and between different strains of the series is due to variation in the reactions induced by the metabolic activity of the organism. These differences vary with the composition of the medium and with the characters of the race or strain studied, but clearly indicate close relationship among the forms.

A. citrisporus von Höhnel. Another form having characters of this group was sent by Dr. Thaxter[46] from excrement of caterpillars. In Czapek's solution agar cultures, this form showed colorless submerged mycelium, and conidial areas at first yellow, then golden, and finally fulvus; stalks 1 to 2 mm. high, up to 20 to 25 μ in diameter, turgid when young, often collapsing in age or when exposed to dry air, septate, with walls thin (1 μ or less mostly), appearing to be studded with fine granules when examined directly (in air) but appearing smooth in liquid except when high magnification and great care are used to demonstrate the abundant pitting; with heads up to 500 μ in diameter; vesicles 30 to 50 μ in diameter, nearly globose, and fertile over nearly the entire surface; sterigmata in one series, 8 to 12 μ by 3 to 4 μ with long, loosely radiating conidial chains. Conidia yellow or golden, then brown, lemon-shaped, 5 to 9 by 5 to 6 μ, rough from irregularly branching ridges of yellow to brown coloring matter between the inner and outer wall.

Sclerotia are occasionally found.

Cultures from excrement of caterpillars by Dr. Roland Thaxter.

[46] Culture received bore the manuscript name *A. chrysospermum*.

A. terricola Marchal. A culture belonging to this group was isolated by Scales[47] from redland soil in Georgia and discussed under the name *A. terricola* Marchal.[48] This culture shows the characteristic morphology of the group but differs in shade of color and in its smaller measurements of stalk, vesicle, and head. It bears about the same relation to *A. tamari* as described above that *A. parasiticus* of Speare does to *A. flavus* as accepted by Wehmer. The color recorded by Marchal, *umbrinus*, readily separates the culture when compared to the colors found in the other members of the group, but the composition of this color shows close relationship to that of *A. tamari* when analyzed in Ridgway's plates.

Scales' culture was submitted to Marchal, who designates the form as *A. terricola* var. *Americana* Marchal, distinguished as follows:

A. terricola var. **Americana** Marchal n. var. "The dimensions of the vesicles 14 to 20 instead of 30 to 50, of the sterigmata 5.6 to 10.5 by 2.2 μ instead of 12 to 15 by 4 to 7 μ; the spores only very delicately verrucose, separate your fungus from *A. terricola*."

A culture nearly related in color and morphology was described by Mrs. Patterson[49] as *A. umbrinus*. The original material and cultures appear to have been lost, and the description lacks details which would decide its exact status.

Nomenclature of the A. tamari series. Kita[50] described as *A. tamari* a culture discovered as a contamination in a Japanese fermented product, tamari-koji. Numerous cultures of American origin show the morphology of *A. tamari* Kita. This identification has been confirmed by conference in which Kita examined a whole series of these strains in culture. The possibility that the organism had been previously described remained for consideration. From its brown color, its identification with *S. castanea* Patterson[51] seemed possible until Mrs. Patterson's exsiccati had been examined and were shown to belong to the *A. niger* group. Upon some media the young heads become definitely orange before becoming brown. This change, together with the pitted stalk and double sterigmata, suggested *A. fulvus* Montagne[52] which was described in connection with silkworm diseases in southern France in 1849, but has not been reported since. Von Höhnel[53], however, described *A. citrisporus* with similar heads from excre-

[47] Scales, F. M. The enzyms of *Aspergillus terricola*. Jour. Biol. Chem. **19**: 259–272. 1914.

[48] Marchal, É. Sur une espèce nouvelle du genre Aspergillus Micheli, *A. terricola*. Rev. Mycol. **15**: 101–3. 1893.

[49] Patterson, F. W. New species of fungi. Bull. Torrey Bot. Club **27**: 284. 1900.

[50] Kita, G. Einige japanische Schimmelpilze. Centralbl. Bakt. II, **37**: 433–452. 1913.

[51] Patterson *loc. cit.*; also exsiccati in pathological collections, U. S. Dept. Agr.

[52] Montagne, C. Plantes cellulaires: Cent. VI, no. 82. Ann. Sci. Nat. Bot. III, **12**: 298. 1849.

[53] Von Höhnel, F. Fragments zur Mykologie, I. Mittheilung. Sitzungsber. K. Akad. Wiss. Wien, Math.-Naturw. Kl. I, **111**: 987–1056. 1902.

ment of caterpillars. The conidia in this form are at first golden yellow, then fulvous, and lemon-shaped instead of globose as given for *A. fulvus*. Dr. Thaxter's culture already described agrees, therefore, closely with *A. citrisporus* as described by von Höhnel. A specimen in the herbarium of the New York Botanical Garden collected by Peck upon excrement of caterpillars at Sandlake, N. Y., appears to be identical with Dr. Thaxter's culture. The characters reported in common for these four strains from caterpillars suggest either identity or the existence of a series of related forms widely distributed and associated with caterpillars. The strain in culture is distinguishable from the forms included under *A. tamari*, although evidently related to it.

The name *A. tamari* Kita is retained here for the group common in food-stuffs and fermentation products, *A. terricola* var. *Americana* Marchal for the soil organism isolated by Scales, and *A. citrisporus* von Höhnel for the caterpillar organism. These are readily separable in culture.

The following paragraphs give specific relations of species and strains of the Aspergilli here under consideration to various culture media.

A. tamari on seed corn. A reversible variation due to corn as a substratum was noticed with strains of *Aspergillus tamari*. We first noticed abnormally large sterigmata and occasionally conidia of this species in exsiccati. For example, a fungus of this type growing on grains of "*Zea Mays*, Waco, Texas, 2–26–1909, Comm. F. Hedges, Pl. Disease Survey," and deposited among the exsiccati of the Department of Agriculture, showed sterigmata with primaries 20 to 30 by 5 to 6 μ and wedge-shaped, secondaries 8 to 9 by 3 to 3.5 μ. S_3 Asp (a strain recovered from canned sardine paste) had on a Czapek agar slant a vesicle measuring 35 μ, primary sterigmata 8 to 14 by 3 to 5 μ, secondaries normal, conidia 5 to 6 μ. Material from this tube was inoculated into a bottle of unsterilized, clean corn. Two weeks later the new culture showed vesicles 100 μ, primary sterigmata 25 to 35 by 5 to 9 μ, secondaries 15 by 3 to 4 μ, conidia 8 to 9 μ. The same observations were made with two other strains of this species. Retransfer from the corn culture to Czapek solution agar produced what is considered typical growth using Czapek as a standard.

Tests for phenolic substances with an aqueous solution of ferric chloride align the *Aspergillus tamari* group closely with the *A. flavus-oryzae* group as to this particular chemical reaction. All strains of these three species which were tested showed a red reaction, varying from brownish red to a rich wine-red. This variation is dependent both on the ability of the individual strain to produce a given quantity of a phenolic substance and on the ingredients of the culture medium. *Aspergillus Wentii* gives, however, a clear yellow color when tested with ferric chloride. The best results were obtained by using a transparent liquid medium[54] from which a sample of

[54] (1) Ordinary household rice extracted in water at 58–60° C. for one hour. (2) Tap water, 1 liter; starch (soluble), 1 per cent; ammonium nitrate, 0.05 percent; K_2HPO_4, 0.05 percent.

from one to several cubic centimeters could be withdrawn with a sterile pipette. These experiments are merely a preliminary step in an investigation of the production of phenols by molds. Dr. J. F. Brewster of the Laboratory of Biological Investigations is at present engaged on this research.

Key to species described from culture.

I. Conidia pitted-areolate (determinable only by oil immersion):
  A. Colonies typically yellow-green at first, shading to yellowish brown in age. *A. flavus-oryzae* series:
    Aerial growth of fertile hyphae only:
      Stalks long, 2 to several mm.: *A. oryzae.*
      Stalks 400 to 600 μ, rarely 1,000 μ: *A. flavus.*
      Stalks 200 to 500 μ, crowded: *A. parasiticus.*
    Aerial growth, both vegetative hyphae and fertile hyphae: *A. effusus.*
  B. Colonies yellow to brown, never green: *A. Wentii.*
II. Conidia bearing brown color-bars:
  Stalks conspicuously pitted, thick-walled:
    Stalks 500 to 1,000 μ long: *A. tamari.*
    Stalks 300 to 600 μ long: *A. terricola.*
  Stalks obscurely pitted, collapsing in age: *A. citrisporus.*

Suggested relationship of species elsewhere described from culture but not identified by us:

A. Belonging to *A. flavus-oryzae* series:
  1. Gigantic and floccose types related to *A. oryzae* (Ahlb.) Cohn:
    Showing transient green: Takahashi's N, near *A. perniciosus.*
    Showing no transient green: Takahashi's D, near *A. giganto-sulphureus.*
  2. Bridging forms intermediate in measurements between typical *A. flavus* and *A. oryzae*:
    A. pseudoflavus Saito.
    A. gymnosardae Yukawa.
  2. Bridging forms as no. 2 with much smaller conidia:
    Takahashi's P, near *A. micro-virido-citrinus* Cost. and Lucet.
B. Probably belonging with *A. tamari*:
  Color butter-yellow, conidia 5 to 7 μ: *S. butyracea* Bainier.
  Color umbrinus, conidia 6 to 9 μ, white sclerotia: *A. umbrinus* Patterson.
C. Pathogenic to man:
  Reported from human ear: *A. flavescens* Wreden.
  Reported from skin lesions: *A. Tokelau* Wehmer.

The following is a bibliography of organisms referred to in this paper or whose relationship to these groups is suggested by the literature. The citations are arranged alphabetically to specific names.

S. albo-lutea Bainier. Bull. Soc. Bot. France **27**: 30. 1880. This was a small pale yellow form, but no further data were given and it has not since been identified.

A. aurantiacus Berkeley is cited by Montagne (Ann. Sci. Nat. Bot. III, **12**: 299. 1849) as having clavate heads. Farlow (Bibliographical Index of North American Fungi **1**, pt. 1, p. 276, issued Sept. 1, 1905) places this organism with *Nematogonium aurantiacum* Desm. Personal examination of

the Curtis collection confirms Farlow's belief that this fungus is not an Aspergillus.

A. aureus Berkeley (Berkeley, M. J.). English Flora **5**: 346. 1836. The golden yellow, elliptical conidia reported by Berkeley suggest *A. citrisporus*, but actual identification from the description is impossible.

S. butyracea Bainier. Bull. Soc. Bot. France **27**: 29. 1880. C. Roumeguère. Fungi Gallici Exsiccati, no. 995. The organism has not since been reported. The material preserved in the Harvard herbarium shows some conidial fruiting fairly typical of *A. niger*, areas which correspond with Bainier's *S. fusca*, and some material which was probably *S. butyracea* but in which details of head structure, mature conidial markings, and measurements were not determinable with certainty. The information obtainable suggests relationship to *A. tamari*.

A. citrisporus von Höhnel. Fragmente zur Mykologie, I. Mittheilung. Sitzungsber. K. Akad. Wiss. Wien, Math.-Naturw. Kl. Abt. I, **111**: 987. 1902. This form was described by von Höhnel from excrement of larvae. A cultural description is given under this name to a form similarly isolated by Thaxter (no. 4186.10). A specimen was collected by Peck at Sandlake, N. Y., from excrement of caterpillars and preserved in the herbarium of the New York Botanical Garden. Another specimen of this species is found in the collection of the Michigan Academy of Science as determined by Kauffman.

S. castanea Patterson. Bull. Torrey Bot. Club **27**: 284. 1900. The color given suggests *A. tamari*, but the exsiccati show that this form was one of the paler forms of *A. niger*.

A. effusus Tiraboschi. Annali di Botanica **7**, fasc. 1: 16. 1908. Name cited without description by Tiraboschi in Atti Terzo Congresso Pellagralogico Italiano, Milano 1906: 139, 142. 1907. This name is accepted for a series of cultures obtained in America and described (page 109).

A. flavescens Wreden. Compt. Rend. Acad. Sci. Paris **65**: 368. 1867. Also St. Petersb. Med. Zeitschr. **13**: 133. 1867. There is no record that the organism of Wreden was cultivated. It was tentatively placed by us (Thom, C., and Church, M. B. Amer. Jour. Bot. **5**: 100. 1918) with *A. nidulans* from the descriptions given, but has been repeatedly cited as a synonym of *A. flavus*. The name was used by Lichtheim (Lichtheim, L. Ueber pathogenen Schimmelpilze. Berliner Klin. Wochenschr. **19**: 128, 147. 1882) and others for a strain certainly belonging to the *A. flavus* series. There is no direct evidence that this usage was based upon positive identification of Wreden's material.

A. flavus Link. Obs. p. 16. 1809. (Link, H. F. Observationes in ordines plantarum naturales. Ges. Naturforsch. Freunde zu Berlin, Magazin 3. 1809, usually cited Link Obs.). The habitat cited by Link was badly dried herbariun specimens. DeBary and Woronin in describing *Eurotium A. flavus* (DeBary, A., and Woronin M., Beiträge zur Morpho-

logie und Physiologie der Pilze, III^te Reihe, 2^te Abt., p. 380) believed their material to be identical with the species of Link. A culture by Brefeld preserved in Rabenhorst, Fungi Europaei Edit. Nov. ser. II, no. 2135, is cited by Wilhelm (Wilhelm, K. A. Beiträge zur Kenntniss der Pilzgattung Aspergillus. Inaug. Diss. Strassburg, p. 59. 1877), and later by Wehmer (Monog. p. 81. 1901). The continuity of the usage of the name *A. flavus* seems therefore well established. We have examined a packet of this material in the collection of the New York Botanical Garden, which is certainly the organism we have described as *A. flavus*. Costantin and Lucet *loc. cit.*, pp. 162, 163) attribute the name *A. flavus* incorrectly to Wilhelm.

A. fulvus Montagne. Plantes cellulaires, Cent. VI, no. 82. Ann. Sci. Nat. Bot. III, **12**: 298. 1849. The description allies this form with the group typified by *A. tamari* in this paper, but *A. fulvus* has never been reported except by Montagne.

A. giganto-sulphureus Saito. Jour. Coll. Sci. Imp. Univ. Tokyo **18**: 48. Pl. 3, figs. 12*a-d*. 1904. While not identified by us positively, the description suggests colonies near D, in Takahashi's series.

A. gymnosardae Yukawa. Jour. Coll. Agr. Tokyo **1**: 362. Pl. 18, figs. 1–7. 1911. This fungus was found by Yukawa under the name "awokabi" and is described by him as essential to the ripening of the tuna-fish preparation, "katsuobushi." The dimensions given are intermediate between those of *A. flavus* and of *A. oryzae*, and closely approximate those of *A. pseudoflavus*. Although we have cultures related to these forms, we have not been able to identify these intermediates.

A. micro-virido-citrinus Costantin & Lucet. Ann. Sci. Nat. Bot. IX, **2**: 158. 1905. The appearances of colonies and measurements of stalks, heads, and spores indicate a form intermediate between *A. flavus* and *A. oryzae* except for its small conidia. The description is very nearly satisfied by Takahashi's culture P.

A. oryzae (Ahlburg) Cohn (Cohn, F. Ueber Schimmelpilze als Gärungserreger. Jahresb. Schles. Ges. für vaterl. Cultur (1883) **61**: 226. Breslau. 1884). Syn., *Eurotium oryzae* Ahlb. The name *E. oryzae* with an incomplete description for the saké organism was published by Korschelt (Korschelt, O. Ueber Saké, das alkoholische Getränk der Japaner. Dingler's Polytechnisches Jour. **230**: 330. 1878) as taken from a letter from "Herr Ahlburg."

A. oryzae var. *basidiferens* Costantin & Lucet. Ann. Sci. Nat. Bot. IX, **2**: 167. 1905. The describers found both secondary and primary sterigmata upon a culture received by them as *A. oryzae*. Without cultivating any other strain, they describe this form as a new variety. Although double sterigmata are not mentioned in Wehmer's description, all strains seen by us have double sterigmata at least under some conditions of culture. Hence the varietal name should be dropped.

Eurotium oryzae Ahlburg. See *A. oryzae* (Ahlb.) Cohn.

A. parasiticus Speare. Hawaiian Sugar Planters' Exp. Sta., Path. and

Physiol. Ser., Bull. **12**: 38. Pls. 3, 4. 1912. Speare found this form parasitic upon the mealy bug of sugar cane in Hawaii. The same form has also been found by us on mealy bugs from Demerara. Experiments, however, with known strains of the *A. flavus* group show that parasitism on the mealy bug is not confined to Speare's strain.

A. perniciosus Inui. Jour. Coll. Sci. Imp. Univ. Tokyo **15**: 473. 1901. Inui recorded a transient green phase in the colonies of this species which is otherwise compared to *A. luchuensis* and *A. Wentii*. In our cultures, *A. luchuensis* and *A. Wentii* have stalks smooth, not pitted as found by Inui in his form. The description suggests certain variant types among Takahashi's cultures such as N.

A. pseudoflavus Saito. Centralbl. Bakt. II, **18**: 34. figs. 15–18. 1907. Syn., *S. pseudoflava* Sacc. Sylloge Fungorum **22**: 1260–1266. The morphology given indicates that *A. pseudoflavus* is one of the intermediate forms which bridge the gap between typical *A. flavus* and *A. oryzae*.

A. siebenmanni Costantin & Lucet. Ann. Sci. Nat. Bot. IX, **2**: 162. 1905. This name is based upon Siebenmann's (Siebenmann, F. Die Fadenpilze *A. flavus, niger, fumigatus, Eurotium repens* und ihre Beziehung zur *Otomycosis aspergillina*. Zeitschr. f. Ohrenheilk. **12**. 1883. Die Schimmelmycosen des menschlichen Ohres. Wiesbaden, 1889) description of an organism from the human ear identified by Siebenmann as *A. flavus*, but regarded by the describers from the description given by Siebermann as a separate species. The data given place the organism correctly in the *A. flavus* group but are not complete enough to separate it.

A. tamari Kita. Centralbl. Bakt. II, **37**: 433. 1913. The strain described in the text was verified as *A. tamari* by Kita. Numerous strains of this group, some of which vary appreciably in cultural detail from the type, have been obtained from purely American as well as from Oriental sources.

A. terricola Marchal (Marchal, Émile). Rev. Myc. **15**: 101. 1893. See also Scales, F. M. Jour. Biol. Chem. **19**: 459. 1914. The culture isolated by Scales was sent by us to Marchal and designated by him *A. terricola* var. *Americana* Marchal in this paper.

A. terricola var. **Americana** Marchal n. var. cultural description Thom and Church. Colonies on Czapek's solution agar from shades near *yellow ocher* (Ridgway XV. 17) when young to *Dresden brown* or *mummy brown* of the same plate (near Saccardo's umbrinus); aerial growth consisting of crowded conidiophores, stalks 300 to 600 μ by 6 to 8 μ, walls pitted; heads radiate; vesicles up to 20 μ in diameter; sterigmata in one series, 7 to 10 μ by 2 to 4 μ; conidia tuberculate from the presence of color bars variously distributed between the outer and inner wall, ovate, from 3 by 5 μ up to 5 by 7 μ or nearly globose, usually about 5.5 μ, occasionally 5 to 8 μ in diameter. Culture by F. M. Scales from Georgia soil. This variety "differs from the type in measurements of vesicle 14 to 20 μ in diameter instead of 40 to 50 μ, in sterigmata 5.6 to 10.5 μ by 2.2 μ instead of 12 to 15 μ by 4 to 7 μ" (Marchal).

A. tokelau Wehmer (Wehmer, C.). Centralbl. Bakt. I, **35**: 140. 1903. The measurements given by Wehmer together with the figures given by Dubreuihl (Dubreuihl, M. W. Jour. Méd. Bordeaux **32**: 312. 1902.) suggest relationship with the *A. flavus* group.

A. umbrinus Patterson (Patterson, Flora W. Bull. Torrey Bot. Club **27**: 284. 1900). The original material of *A. umbrinus* appears to be lost. Although probably related to *A. Wentii* or to *A. tamari*, no material identifiable by this description has been seen.

A. variabilis Gasperini (Gasperini, G. Atti Soc. Toscana Nat. Sci. Pisa, Mem. 8, fasc. 2: 326. 1887). From the description, this was probably some strain of the *A. flavus* group. Both large and small heads were found in the same colony; the sterigmata were simple or double; the conidia have the range of form and size characteristic of the group. Gasperini does not appear to have known *A. flavus*.

A. Wehmeri Costantin & Lucet. Ann. Sci. Nat. Bot. IX, **2**: 162. 1905. The name is proposed by Costantin and Lucet for the organism of Brefeld and Wehmer described as *A. flavus* Link by them and so used in this paper. The uncertainties in the identification of Link's species do not seem important enough to justify the change of name. *A. Wehmeri* is to be regarded as a synonym of *A. flavus*.

A. Wentii Wehmer. Centralbl. Bakt. II, **2**: 150. 1895. See also Wehmer, Die Pilzgattung Aspergillus, etc., in Mém. Soc. Phys. d'Hist. Nat. Genève **33**: 2. Part. 4: 119. 1901. The culture described in the text was received from Amsterdam and appears to be the original strain investigated by Wehmer.